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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 645097C:ANB:VNB	FOR FURTHER ACTION		See Form PCT/IPEA/416
International application No. PCT/AU2004/001675	International filing date (day/month/year) 26 November 2004	Priority date (day/month/year) 28 November 2003	
International Patent Classification (IPC) or national classification and IPC Int. Cl. C12N 15/24 (2006.01) C12N 15/38 (2006.01) C12Q 1/68 (2006.01)			
Applicant THE UNIVERSITY OF SYDNEY et al			

- This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
- This REPORT consists of a total of 7 sheets, including this cover sheet.
- This report is also accompanied by ANNEXES, comprising:
 - ☒ (sent to the applicant and to the International Bureau) a total of 7 sheets, as follows:
 - ☐ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
 - ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
 - ☐ (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

- This report contains indications relating to the following items:

<input checked="" type="checkbox"/> Box No. I	Basis of the report
<input type="checkbox"/> Box No. II	Priority
<input checked="" type="checkbox"/> Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
<input type="checkbox"/> Box No. IV	Lack of unity of invention
<input checked="" type="checkbox"/> Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
<input type="checkbox"/> Box No. VI	Certain documents cited
<input type="checkbox"/> Box No. VII	Certain defects in the international application
<input checked="" type="checkbox"/> Box No. VIII	Certain observations on the international application

Date of submission of the demand 28 September 2005	Date of completion of this report 03 March 2006
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer Jamie Turner Telephone No. (02) 6283 2071

Box No. I Basis of the report

1. With regard to the language, this report is based on:

☒ The international application in the language in which it was filed☐ A translation of the international application into _____, which is the language of a translation furnished for the purposes of:☐ international search (under Rules 12.3(a) and 23.1 (b))☐ publication of the international application (under Rule 12.4(a))☐ international preliminary examination (Rules 55.2(a) and/or 55.3(a))2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:☐ the international application as originally filed/furnished☒ the description:

pages 1, 3-44 as originally filed/furnished

pages* 2 received by this Authority on 28 September 2005 with the letter of 28 September 2005

pages* received by this Authority on with the letter of

☒ the claims:

pages as originally filed/furnished

pages* as amended (together with any statement) under Article 19

pages* 45-50 received by this Authority on 28 September 2005 with the letter of 28 September 2005

pages* received by this Authority on with the letter of

☒ the drawings:

pages as originally filed/furnished

pages* 1/7-7/7 received by this Authority on 2 March 2005 with the letter of 2 March 2005

pages* received by this Authority on with the letter of

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.3. ☐ The amendments have resulted in the cancellation of:☐ the description, pages☐ the claims, Nos.☐ the drawings, sheets/figs☐ the sequence listing (*specify*):☐ any table(s) related to the sequence listing (*specify*):4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).☐ the description, pages☐ the claims, Nos.☐ the drawings, sheets/figs☐ the sequence listing (*specify*):☐ any table(s) related to the sequence listing (*specify*):

* If item 4 applies, some or all of those sheets may be marked "superseded."

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos: 9 (completely) and 23, 24, 26, 27, 33, 40-42, and 45-51 (all partially)

because:

☐ the said international application, or the said claims Nos.

relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos.
are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. 9 (completely) and 23, 24, 26, 27, 33, 40-42, and 45-51 (all partially)
are so inadequately supported by the description that no meaningful opinion could be formed (*see Box VIII*)

☐ no international search report has been established for said claim Nos.

☐ A meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ Furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ Furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ Pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13ter.1(a) or (b) and 13ter.2.

☐ A meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-bis of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.

☐ See Supplemental Box for further details.

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-22, 25, 28-32, 34-39, 43-44, 52 (completely) and 23-24, 26-27, 33, 40-42, 45-51 (all partially)	YES
	Claims	NO
Inventive step (IS)	Claims 1-22, 25, 28-32, 34-39, 43-44, 52 (completely) and 23-24, 26-27, 33, 40-42, 45-51 (all partially)	YES
	Claims	NO
Industrial applicability (IA)	Claims 1-22, 25, 28-32, 34-39, 43-44, 52 (completely) and 23-24, 26-27, 33, 40-42, 45-51 (all partially)	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

In general, the present application relates to a IL-10 homologue which is expressed by human cytomegalovirus during the latent phase of viral infection.

The following citations raised in the ISR were:

D1: Jenkins, C. et al (2002) "Human cytomegalovirus UL111.5A-region transcripts are expressed during both experimental and natural latent infection of myeloid cells" *3rd College of Health Sciences and Medical Foundation Research Conference: From Cell to Society 3*, 18-SEPT-2002 to 19-SEPT 2002, Blue mountains, Australia, e-poster/mini-poster number 22-9.

D2: Xu, Z-G et al (2001) "The latency pattern of Epstein-Barr virus infection and viral IL-10 expression in cutaneous natural killer/T-cell lymphomas" *British Journal of Cancer* 84(7): 920-925.

D3: Miyazaki, I et al (1993) "Viral Interleukin 10 Is Critical for the Induction of B Cell Growth Transformation by Epstein-Barr Virus" *The Journal of Experimental Medicine* 187: 439-447

D4: Jenkins, C. et al (2004) "A Novel Transcript with Homology to Human Interleukin-10 Is Expressed during Latent Human Cytomegalovirus Infection" *Journal of Virology* 78(3): 1440-1447.

D2 and D3 are not considered relevant to the claim set of 28 September 2005.

D4 does not form part of the prior art. However, if the priority of the application is invalid this document may become relevant.

Continued in Supplemental Box

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 9 (completely) and claims 23, 24, 26, 27, 33, 40-42, and 45-51 (partially) are not fully supported by the specification. These claims encompass or make use of a large number of possible ligands. The specification only supports ligands which are derivatives of the cmvIL-10 homologue peptide or its coding sequence and ligands such as antibodies which have been prepared using the cmvIL-10 homologue peptide.

Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:
 - a. type of material
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material
 - ☒ on paper
 - ☒ in electronic form
 - c. time of filing/furnishing
 - ☐ contained in the international application as filed
 - ☒ filed together with the international application in electronic form
 - ☐ furnished subsequently to this Authority for the purposes of search and/or examination
 - ☐ received by this Authority as an amendment* on
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

* If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box V

D1 is clearly the most relevant prior art to this application. D1 teaches the isolation of an UL111.5A-region transcript which is expressed during latent cmv infection and that this transcript is a splice variant of cmvIL-10. The document teaches that the splice variant differs from cmvIL-10 in that intron 2 remains unspliced.

D1 does not explicitly teach the nucleic acid sequence of the splice variant. While the skilled person could determine, from D1, the 3' stop site, the 5' start site is not disclosed therein nor could it be necessarily elucidated (especially in the absence of any described primer sequences). Further, the skilled person could not predict the length of the transcript. Lastly, D1 does not explicitly disclose the splicing pattern of the transcript. In the absence of this information, the translated amino acids may bear little resemblance to those claimed in the present application.

In light of the forgoing, claims 1-8 and 10-52, which are limited to specific amino acid and nucleotide sequence, must be considered both novel and inventive in view of D1.

Industrial applicability is acknowledged.

the MIE region CLTs have not yet been defined. Furthermore, few studies have sought to assess viral gene expression during latency.

The present invention relates to the surprising discovery that a region of the genome of a virus of the herpesviridae group is expressed during the latent phase of infection.

Summary of the Invention

According to a first embodiment of the present invention there is provided a purified nucleic acid sequence encoding a homologue of human interleukin 10 (IL-10), wherein said IL-10 homologue is expressed during the latent phase of infection by a virus of the herpesviridae group.

The nucleic acid sequence may be as set forth in SEQ ID NO:1, or a fragment or variant thereof.

According to a second embodiment of the present invention there is provided a human interleukin 10 (IL-10) homologue polypeptide, wherein said IL-10 homologue is expressed during the latent phase of infection by a virus of the herpesviridae group. The IL-10 homologue may be encoded by the nucleic acid sequence in accordance with the first embodiment of the invention. The IL-10 homologue may be the product of alternative splicing of the primary RNA transcript. For example, the IL-10 homologue may be from the UL111.5A region of the cytomegalovirus genome. The IL-10 homologue may have the amino acid sequence as set forth in SEQ ID NO:10, or the amino acid sequence as set forth in SEQ ID NO:10 including one or more conservative amino acid substitutions.

The virus of the herpesviridae group may be selected from the group consisting of: Epstein-Barr virus, human herpesvirus (HHV)-6, HHV-7, HHV-8, varicella zoster virus, herpes simplex type 1 and type 2 virus and cytomegalovirus. Moreover, the virus may be cytomegalovirus.

In a third embodiment, the present invention provides a vector comprising a nucleic acid sequence in accordance with the first embodiment of the invention.

In a fourth embodiment, the present invention provides a recombinant host cell comprising the nucleic acid in accordance with the first embodiment of the invention or the vector in accordance with the third embodiment of the invention.

In a fifth embodiment, the present invention provides a recombinant host cell capable of expressing the polypeptide or variant or fragment thereof of the second embodiment of the invention.

Claims

1. A purified nucleic acid sequence encoding a homologue of human interleukin 10 (IL-10), wherein said IL-10 homologue is expressed during the latent phase of infection by a virus of the herpesviridae group, and wherein said nucleic acid sequence is as set forth in SEQ ID NO:1.

2. The nucleic acid of claim 1 wherein the virus of the herpesviridae group is selected from the group consisting of: Epstein-Barr virus, human herpesvirus (HHV)-6, HHV-7, HHV-8, varicella zoster virus, herpes simplex type 1 and type 2 virus and cytomegalovirus.

3. An isolated homologue of human interleukin 10 (IL-10) polypeptide, wherein said IL-10 homologue is expressed during the latent phase of infection by a virus of the herpesviridae group, and wherein said IL-10 homologue has the amino acid sequence as set forth in SEQ ID NO:10, or the amino acid sequence as set forth in SEQ ID NO:10 including one or more conservative amino acid substitutions.

4. The IL-10 homologue of claim 3, wherein said homologue is the product of alternative splicing of the primary RNA transcript.

5. The IL-10 homologue of claim 3 or 4, wherein said IL-10 homologue is from the UL111.5A region of the cytomegalovirus genome.

6. A vector comprising a nucleic acid sequence in accordance with either one of claims 1 or 2, or a nucleic acid encoding the polypeptide of any one of claims 3 to 5.

7. A recombinant host cell comprising the nucleic acid sequence in accordance with either one of claims 1 or 2 or the vector in accordance with claim 6.

8. A recombinant host cell capable of expressing the polypeptide of any one of claims 3 to 5.

9. An isolated ligand that selectively binds to the polypeptide of any one of claims 3 to 5.

10. The ligand of claim 9, wherein said ligand is an antibody.

11. A method of identifying a compound that interacts with the polypeptide of any one of claims 3 to 5, the method comprising the steps of:

(a) contacting a candidate compound with the polypeptide under conditions suitable to permit interaction of the candidate compound to the polypeptide thereof; and

(b) detecting the interaction between the candidate compound and the polypeptide.

12. The method of claim 11, wherein said interaction is detected by adding a labelled substrate and measuring a change in the labelled substrate.

13. A method of identifying a compound that binds to the polypeptide of any one of claims 3 to 5, the method comprising the steps of:

- (a) contacting a candidate compound with the polypeptide; and
- (b) assaying for the formation of a complex between the candidate compound and the polypeptide.

14. The method of claim 13, wherein said assay for the formation of a complex be selected from the group consisting of: a competitive binding assay, a two-hybrid assay or an immunoprecipitation assay.

15. A method of screening for a compound that modulates the activity of the polypeptide of any one of claims 3 to 5, the method comprising the steps of:

- (a) contacting the polypeptide with a candidate compound under conditions suitable to enable interaction of the candidate compound to the polypeptide; and
- (b) assaying for activity of the polypeptide.

16. The method of claim 15, wherein said assay for activity of the polypeptide comprises adding a labelled substrate and measuring a change in the labelled substrate.

17. A method of diagnosing a disease state, or predisposition to a disease state, in a subject, the method comprising the steps of:

- (a) obtaining a biological sample from the subject; and
- (b) assaying for expression of the polypeptide of any one of claims 3 to 5 in the sample.

18. The method of claim 17, wherein said assay for the expression of the polypeptide comprises contacting the biological sample with a compound capable of interacting with the polypeptide such that the interaction can be detected.

19. The method of claim 17 or 18, wherein the compound capable of selectively interacting with the polypeptide is an antibody or fragment thereof.

20. A method of identifying an agent which is an inhibitor of infection by a virus of the herpesviridae group, the method comprising contacting a cell or cell extract with one or more candidate agents, determining whether there is a change in the activity of a polypeptide of any one of claims 3 to 5 and thereby determining whether the agent is an inhibitor of a virus of the herpesviridae group.

21. The method of any one claims 11 to 20, wherein said viruses of the herpesviridae group are selected from the group consisting of: Epstein-Barr virus, human herpesvirus (HHV)-6, HHV-7, HHV-8, varicella zoster virus, herpes simplex type 1 and type 2 and cytomegalovirus.

22. A method of identifying an agent suitable for use in the treatment or prevention of a disease state in a subject, the method comprising:

- (a) obtaining a biological sample from the subject,
- (b) contacting the sample with a candidate agent,
- 5 (c) determining whether there is a change in the activity of the polypeptide of any one of claims 3 to 5, and
- (d) thereby determining whether the agent is suitable for use in the treatment of the disease state.

23. A method for treating or preventing a disease state in a subject, the method
10 comprising administering to the subject a therapeutically effective amount of the ligand of claim 9 or 10 or a compound identified by the method of any one of claims 11 to 22.

24. A kit comprising the nucleic acid sequence in accordance with either one of claims 1 or 2 or the polypeptide of any one of claims 3 to 5, or the ligand of claim 9 or 10.

25. The kit of claim 24, wherein the ligand is an antibody.

15 26. A method for screening a subject for infection by a virus of the herpesviridae group, the method comprising:

- (a) obtaining a biological sample from said subject;
- (b) contacting said sample with the ligand of claim 9 or 10, and
- (c) detecting the presence of ligand selectively bound to the polypeptide of any
20 one of claims 3 to 5.

27. The method of claim 26, wherein the biological sample is a plasma or cell sample.

28. A method for screening a subject for infection by a virus of the herpesviridae group, the method comprising:

- 25 (a) obtaining a biological sample from said subject;
- (b) contacting said biological sample from said subject with the nucleic acid sequence of either one of claims 1 or 2; and
- (c) detecting the presence or absence of hybridisation between the nucleic acid sample of said biological subject and the nucleic acid sequence of either one of claims 1
30 or 2.

29. A method for screening a biological sample for infection by a virus of the herpesviridae group, the method comprising:

- (a) obtaining a biological sample from said sample;
- (b) contacting said biological sample from said subject with the nucleic acid
35 sequence of either one of claims 1 or 2; and

(c) detecting the presence or absence of hybridisation between the nucleic acid sample of said biological sample and the nucleic acid sequence of any one of claims 1 to 3.

30. The method of claim 28 or 29, wherein the nucleic acid is capable of selectively hybridising to the nucleic acid encoding the IL-10 homologue expressed during the latent phase of infection by a virus of the herpesviridae group.

31. The method of any one of claims 28 to 30, wherein the nucleic acid sequence corresponds to any one of SEQ ID Nos:2 to 9.

32. An isolated nucleic acid, wherein the nucleic acid sequence corresponds to any one of SEQ ID Nos:2 to 9.

33. A method for screening a biological sample for infection by a virus of the herpesviridae group, the method comprising:

- (a) contacting said biological sample with the ligand of claims 9 or 10, and
- (b) detecting the presence of the ligand selectively bound to the polypeptide of any one of claims 3 to 5.

34. The method of claim 33, wherein said ligand is an antibody.

35. The method of claim 33 or 34, wherein the sample is selected from the group consisting of: blood, bone marrow or organ(s) or spinal fluid.

36. The method of any one of claims 30 to 31 or 33 to 35, wherein the sample is intended to be used in a subject selected from the group consisting of: transplant recipients (bone marrow, stem cell or solid organ), subjects undergoing immunosuppression therapy and immunocompromised subjects.

37. The method of claim 36, wherein the immunocompromised subject is a subject suffering from acquired immune deficiency syndrome (AIDS) or diagnosed as infected with human immunodeficiency virus (HIV).

38. A method of immunosuppression in a subject, said method comprising administering a therapeutically effective amount of the polypeptide of any one of claims 3 to 5.

39. The method of any one of claims 22 to 31 or 33 to 38, wherein the virus of the herpesviridae group is selected from the group consisting of: Epstein-Barr virus, human herpesvirus (HHV)-6, HHV-7, HHV-8, varicella zoster virus, herpes simplex type 1 and type 2 and cytomegalovirus.

40. A vaccine, wherein said vaccine comprises a nucleic acid molecule of either one of claims 1 or 2, or a polypeptide of any one of claims 3 to 5, or a ligand of claim 9 or 10, together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

41. A method for inducing an immune response in a vertebrate against disease associated with infection by a virus of the herpesviridae group, comprising administering to said vertebrate an immunologically effective amount of the polypeptide of any one of claims 3 to 5, or a ligand of claim 9 or 10, or a vaccine of claim 40.

5 42. A method for the treatment and/or prophylaxis of disease associated with infection by a virus of the herpesviridae group in a vertebrate, wherein said method comprises administering a therapeutically effective amount of the polypeptide of any one of claims 3 to 5, or a ligand of claim 9 or 10, or the vaccine of claim 40.

10 43. The method of claim 41 or 42, wherein the polypeptide or ligand is simultaneously or sequentially administered with cytokines.

44. The method of claim 43, wherein the cytokines are selected from the group consisting of: G-CSF, GM-CSF and interleukins.

45. A method of cleansing a biological sample of infection by a virus of the herpesviridae group, the method comprising:

- 15 (a) contacting said biological sample with the ligand of claim 9 or 10,
(b) detecting the presence of the ligand bound to a cell expressing the polypeptide of any one of claims 3 to 5, and
(c) removing said cell to which said ligand binds.

20 46. The method of claim 45, wherein the detection step (b) is an intracellular staining assay.

47. The method of claim 46, wherein the cells identified are then be removed from a mixed cell population by flow cytometry.

48. The method of any one of claims 17 to 31, 33 to 39 or 41 to 47, wherein the disease state is one arising from infection by a virus of the herpesviridae group.

25 49. The method of claim 48, wherein the disease is selected from the group consisting of: Epstein-Barr virus, human herpesvirus (HHV)-6, HHV-7, HHV-8, varicella zoster virus, herpes simplex type 1 and type 2 and cytomegalovirus.

50. A cleansed biological sample prepared in accordance with the method of any one of claims 45 to 49.

30 51. A method of diagnosis of infection of a subject by a virus of the herpesviridae group, the method comprising:

- (a) contacting a biological sample of the subject with the ligand of claim 9 or 10,
(b) detecting the presence of the ligand thereof selectively bound to the polypeptide of any one of claims 3 to 5.

52. A method of diagnosis of infection of a subject by a virus of the herpesviridae group, the method comprising:

- (a) obtaining a biological sample from said subject;
- (b) contacting said biological sample from said subject with the nucleic acid
5 sequence of either one of claims 1 or 2; and
- (c) detecting the presence or absence of hybridisation between the nucleic acid sample of said biological sample and the nucleic acid sequence either one of claims 1 or 2.